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Cellulase Activity in Meiobenthos in Wetlands

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Abstract

To validate the involvement of meiobenthos in cellulose breakdown in wetlands, meiobenthos were collected from the sediments of Lake Furen and the Biwase River in Hokkaido prefecture, the Kako River in Hyogo prefecture, and the Chinai River in Shiga prefecture. Cellulase activities of the meiobenthos were measured by cellulose zymographic analysis using SDS-PAGE gels containing 0.5% carboxymethyl cellulose. The results showed that most of the Turbellaria, Nematoda, Harpacticoida and Oligochaeta species exhibited cellulase activity. The molecular sizes of the cellulase-active bands of the sediments in Lake Furen, the Biwase River, and the Chinai River coincided with those of meiobenthos. The findings suggest that meiobenthos might play a major function in cellulose breakdown in these wetlands. This paper is the first to report cellulase activity in meiobenthos and that they are possibly involved in the breakdown of cellulose in wetlands.

KEY WORDS: cellulase・ cellulose・ meiobenthos・ sediment・wetland

Introduction

Cellulose, the most abundant organic matter on earth, is a high molecular weight substance consisting of glucose residues bound by β-1,4 linkages, unlike starch, another glucan consisting of a α-1,4 linked glucose residues. Cellulose is resistant to enzyme degradation [1, 2]. However, cellulose can be degraded by specific enzymes collectively named cellulases [3].

Fungi and bacteria as well as symbiotic protozoa in herbivorous animals and termites have been studied as known consumers of cellulose [4]. Recently, an intrinsic cellulase gene was isolated from termite [5], and a variety of intrinsic cellulase genes were identified from various animals, including beetle [6], nematode [7, 8], abalone [9], mussel [10], sea urchin [11], and brackish-water clam [12].

Recent stable isotopic analysis showed that a brackish water clam *Corbicula japonica* consumes land-derived organic materials mainly composed of cellulose [13]. Identification of the intrinsic cellulase gene and immunological detection of the enzyme protein in *C. japonica* strongly suggest that *C. japonica* plays an important role in the process of degradation of cellulose in rivers [14].

Besides macrobenthos such as *C. japonica*, a group of small animals called meiobenthos also inhabits the sediments of aquatic areas. Meiobenthos are defined as animals that pass through a 1-mm mesh filter and are known to be composed of a variety of fauna corresponding to 22 phyla [15].

In the present study, we attempted to validate the role of meiobenthos in the process of breakdown of cellulose in wetlands. We chose Lake Furen and the Biwase River located in the subarctic area, since they were reported to be the wetlands demonstrating the highest cellulase activities in Japan [16]. On the other hand, we chose the Kako River and the Chinai River as typical rivers in temperate area. We also expected the difference in distribution of meiobenthos between the Kako River and the Chinai River, because the Chinai River is a fresh water river. We report that meiobenthos have cellulase activity and possibly play substantially important roles in the cellulose degradation process in the sediments of some wetlands.

Materials and Methods

Materials

We collected sediments from Lake Furen and the Biwase River in Hokkaido prefecture, the Kako River in Hyogo prefecture, and the Chinai River in Shiga prefecture. River sediments from all rivers were collected in the wetland within 50 m from the river mouth. From each site, we collected sediment samples from a 5-cm depth at low tide from May 2006 to October 2007. The sediment samples were transported to the laboratory in Kyoto University at 4°C, and meiobenthos were recovered in the fraction that included material small enough to pass through a 1mm-mesh filter but too large to pass through a 40 μm-mesh filter. These meiobenthos were classified under microscopic observation according to Higgins and Thiel [15]. About 500 g of the sediments were filtered through 1 mm-mesh. Sediment samples that were not filtered were designated "total sediment fraction," while sediments less than 1 mm and larger than 40 μm were collected and designated "meiobenthos fraction."

Measurement of cellulase activity

Meiobenthos were separated from the sediments by using a pair of tweezers under the microscope (Olympus, S2X12, Tokyo), and each single meiobenthos was homogenized with 20 μl of phosphate-buffered saline (PBS) containing 140 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO4, and 1.5 mM KH_2PO_4 (pH 7.4) to prepare a meiobenthos extract. The total sediment and meiobenthos fractions were homogenized with 1.5-fold volume of

PBS, and the supernatants were obtained by centrifugation at 10,000 × *g* for 10 min. Ten microgram of the supernatant was applied on cellulase zymographic analysis. Cellulase zymographic analysis was performed as described previously by using 7.5% or 10% SDS-PAGE gel containing 0.5% carboxymethyl cellulose (CMC, Sigma, St Louis, MO, US). After electrophoresis, the gels were soaked in 10 mM acetate buffer (pH 5.5) containing 0.1% TritonX-100 for 30 min to remove SDS from the gels. The gels were transferred to 10 mM acetate buffer (pH 5.5), incubated at 37°C overnight, and then stained with 0.1% Congo Red. The gels were destained using 1 M NaCl. The active bands were detected as non-stained bands. Unless otherwise specified, special grades of reagents were commercially obtained from Nacalai Tesque (Kyoto). Protein concentration was measured according to the method of Bradford (17).

Results

Distribution of meiobenthos

Oligochaeta species were the dominant meiobenthos in Lake Furen, where the sediments are mainly composed of sand. In addition, a variety of Turbellaria, Nematoda, Harpacticoida species were also observed. Oligochaeta species were also dominant in the Chinai River, where the sediments were, like in Lake Furen, mainly composed of sand.

In the Biwase and Kako Rivers, where the sediments were mainly composed of clay, Nematoda and Harpacticoida species were dominantly observed.

Cellulase activity of sediments and meiobenthos

Cellulase activity in meiobenthos extracts, total sediment fractions, and meiobenthos fractions were measured by cellulose zymography.

Cellulase activity bands of the total sediment fraction were detected between 32.5 kDa and 47.5 kDa in samples from Lake Furen, which coincided with those of the meiobenthos fraction (lanes 1 and 2 in Fig. 1). Oligochaeta species assayed by using a single animal showed an active band corresponding to 32.5 kDa, which is the band size observed for the total sediment fraction and the meiobenthos fraction (lane 3 in Fig. 1). Different species of Nematoda showed active bands of different molecular sizes, as shown in lanes 4–7 of Fig. 1. Interestingly, common active bands at 25 kDa were detected for all the Nematoda species. Various active bands were also detected for

Fig.1

Turbellaria species (lane 8 in Fig. 1), but their sizes differed from those of Nematoda species.

Figure 2 shows the cellulase activity in sediment samples corresponding to approximately 5 mg per one lane from the Biwase River. Intensive active bands were observed, especially above 47.3 kDa in the total sediment fraction and the meiobenthos fraction (lanes 1 and 2 in Fig. 2), and the active band patterns were nearly identical. Harpacticoida species, the dominant organisms of meiobenthos in the Biwase River, also exhibited remarkably intensive activity bands of above 47.3 kDa (lane 3 in Fig. 2). Fig.2

Figure 3 shows the active bands of the samples from the Chinai River. Faint active bands were observed at 25 kDa and between 47.5 kDa and 62 kDa in the total sediment fraction and the meiobenthos fraction (lanes 1 and 2 in Fig. 3). Bands with nearly the same activity were observed for Oligochaeta species, as shown in lane 3. Fig.3

Figure 4 shows the active bands in the Kako River samples. In the total sediment fraction and the meiobenthos fraction, faint bands of less than 25 kDa were observed (lanes 1 and 2 in Fig. 4). In lane 3, active bands of Harpacticoida species were observed at approximately 47.5 kDa, which did not coincide with the active bands in the total sediment fraction and the meiobenthos fraction. Fig.4

Discussion

Cellulase activities were detected in the extracts of the meiobenthos collected from all the sampling sites examined, suggesting that meiobenthos may be involved in the breakdown of cellulose in the sediments. Interestingly, 25 kDa active bands were commonly detected in the extracts of morphologically distinct Nematoda species collected from Lake Furen (lanes 4–7 in Fig. 1), suggesting that Nematoda species possibly share a related cellulase gene. On the other hand, Oligochaeta species collected from Lake Furen and the Chinai River were morphologically distinct and the band sizes of the cellulase were also different (compare lane 3 in Fig. 1 and lane 3 in Fig. 3).

The International Collaborative Research on the Management of Wetland Ecosystem of the National Institute for Environmental Studies [16] reported the outstanding strong cellulase activities of sediments collected from Lake Furen and the Biwase River in Hokkaido among many Japanese wetlands tested. The report attributed the strong cellulase activity in the sediments of Lake Furen and the Biwase River to microorganisms including bacteria and fungus. However, the active bands in cellulose zymographic analyses showed that the position of the active bands coincided with the sediment fractions and the extracts of meiobenthos (Figs. 1 and 2), which supported the hypothesis that meiobenthos might be involved in the breakdown of cellulose in Lake Furen and the Biwase River.

Because recent molecular biological studies suggest the endogenous origin of the cellulase genes in aquatic invertebrates [9, 11, 12], cellulase genes of meiobenthos could be encoded in the DNA of meiobenthos themselves. We are now trying to clone the cellulase genes of meiobenthos to validate the possibility of its endogenous origin.

As shown in Fig. 3, active bands of the total sediment fraction and the meiobenthos fraction coincided with those of Oligochaeta species from the Chinai River. On the other hand, active bands of the total sediment fraction and the meiobenthos fraction did not coincide with those of the Harpacticoida species from the Kako River, as shown in Fig. 4. Thus, the origin of cellulase could not be concluded to be the meiobenthos in the case of Kako River sediment. Further studies are required to evaluate the contribution of meiobenthos to the breakdown of cellulose in wetlands in the temperate area.

The contribution of termites to the breakdown of cellulose in the forests of tropical zones is assumed to correspond to 80% the total cellulose breakdown in this area [18]. Like termites, meiobenthos could be major consumers of cellulose, especially in some wetlands in Hokkaido, because cellulase activity of meiobenthos in Lake Furen and the Biwase River were detected at 4°C (data not shown), which is a temperature at which the growth of bacteria and fungi would be suppressed. Therefore, it seems probable that meiobenthos would play important roles in cellulose degradation especially in low temperature environments like wetlands in Hokkaido.

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Figure legends

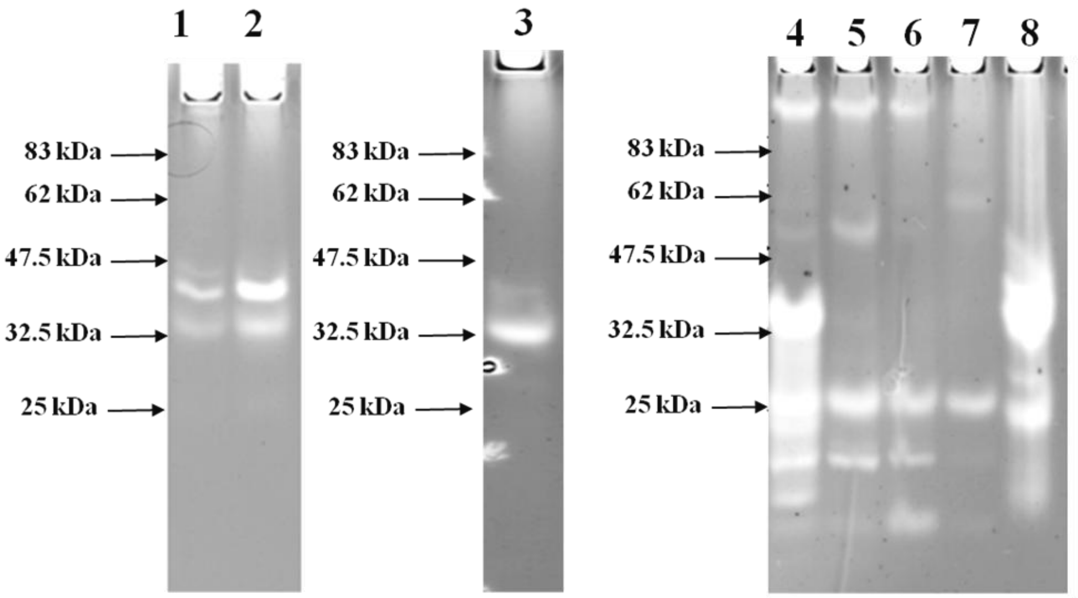
Fig. 1. Cellulase activities in Lake Furen. Activities were detected in 10% SDS-PAGE gel containing 0.5% carboxymethyl cellulose. The positions for molecular mass marker proteins are shown by arrows. Lanes: 1, total sediment fraction; 2, meiobenthos fraction; 3, Oligochaeta species; 4–7, morphologically distinct species of Nematode; 8, Turbellaria species.

Fig. 2. Cellulase activities in the Biwase River. Ten percent SDS-PAGE gel containing 0.5% carboxymethyl cellulose was used for the detection of cellulase bands of the sediments, while 7.5% gel was used for Harpacticoida species. The positions for molecular mass marker proteins are shown by arrows. Lanes: 1, total sediment fraction; 2, meiobenthos fraction; 3, Harpacticoida species.

Fig. 3. Cellulase activities in the Chinai River. Activities were detected in 10% SDS-PAGE gel containing 0.5% carboxymethyl cellulose. The positions for molecular mass marker proteins are shown by arrows. Lanes: 1, total sediment fraction; 2, meiobenthos fraction; 3, Oligochaeta species.

Fig. 4. Cellulase activities in the Kako River. Activities were detected in 10% SDS-PAGE gel containing 0.5% carboxymethyl cellulose. The positions for molecular mass marker proteins are shown by arrows. Lanes: 1, total sediment fraction; 2, meiobenthos fraction; 3, Harpacticoida species.

(Figure 1)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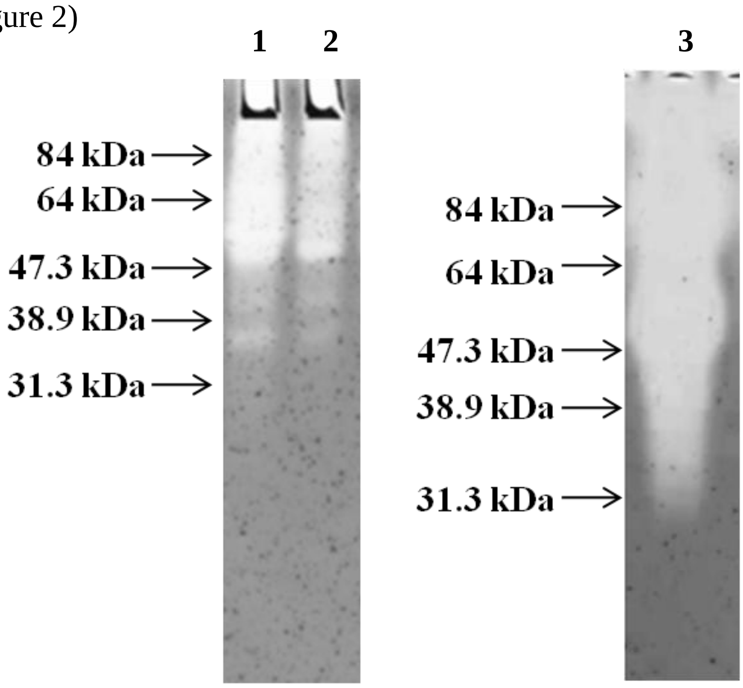
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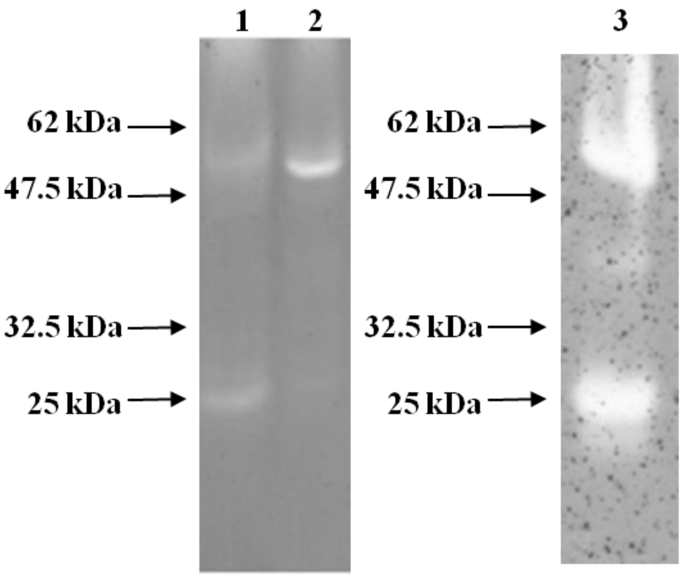

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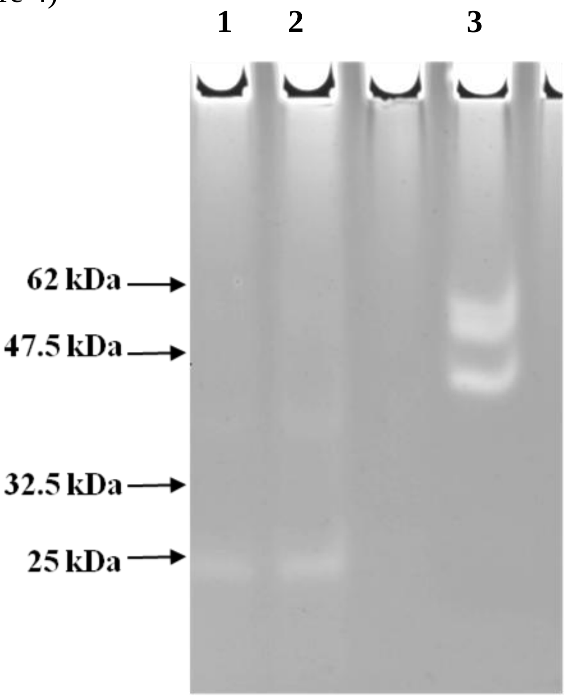

和文要旨

湿地帯に生息するメイオベントスのセルラーゼ活性

豊原治彦，朴　煐華，土屋佳奈子，劉　文（京大院農）

湿地帯におけるセルロース分解に関わるメイオベントスの役割を明らかにする目的で，琵琶瀬川と風蓮湖（北海道），知内川（滋賀県），加古川（兵庫県）の底泥に生息するメイオベントスのセルラーゼ活性を測定し，ほとんどのメイオベントスに活性を認めた。とくに風蓮湖，琵琶瀬川および知内川ではザイモグラフィー分析により，底泥とメイオベントスの活性バンドのサイズが一致したことから，これらの湿地帯においてはセルロース分解にメイオベントスが主要な役割を果たしていると考えられた。